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Molecular identification of *Enterocytozoon bieneusi* and *Encephalitozoon* spp. in immunodeficient patients in Ahvaz, Southwest of Iran

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ABSTRACT

Microsporidia are often considered as an opportunistic infection in patients with impaired immune systems such as transplant recipients and patients with acquired immune deficiency syndrome (AIDS). Due to the increasing prevalence of parasitic infections and immunodeficiency diseases; the aim of the study is to evaluate molecular identification of *Enterocytozoon bieneusi* and *Encephalitozoon* spp. in immunodeficient patients in Ahvaz, southwest of Iran. At first, 310 stool samples were collected from patients with immunodeficiency. The specimens were stained by modified trichrome (weber) and were examined microscopically. The extracted DNA samples were evaluated by multiplex/nested PCR method. The products of multiplex/nested PCR were explored by RFLP method using the restriction enzyme of Mnl1. Of 310, 93 samples were suspected positive for microsporidia by the staining. Also, of 310, 88 samples were positive by the multiplex/nested-PCR test that 62 samples were positive for *E. bieneusi* as well as 26 were detected as *Encephalitozoon* species that including 3 *E. cuniculi*, 19 *E. intestinalis* and 4 *E. hellem*. Of 62 *E. bieneusi,* 45, 16 and 1 were detected as genotype D, M and WL11, respectively. Also, Of 3 *E. cuniculi,* 1 and 2 cases were identified as genotype I and II, respectively. All *E. hellem* samples were included genotype 1A. Our findings revealed a relatively high prevalence of microsporidia species in immunodeficient patients. The highest risk of this infection is at individuals with impaired immune systems that it can be life-threatening in people with immune system dysfunction. It is essential that the high-risk people should be receiving the information about the risk of direct contact with infected individuals and animals.

1. Introduction

Microsporidia are obligate intracellular pathogens. The parasite can infect a wide range of vertebrate and invertebrate hosts among all over the world (Mirjalali et al., 2015). Initially, the microorganisms assumed as protozoa, but in the 1990s, according to phylogenetic and molecular analysis, proofs showed relationship of these organisms and Fungi (Pirestani et al., 2013). The phylum microsporidia is including about 150 genera and 1200 species. *Encephalitozoon* species (*E. hellem, E. intestinalis* and *E. cuniculi*) and *Enterocytozoon bieneusi* (*E. bieneusi*) are the most frequent causes of human infections (Sak et al., 2010).

Microsporidia are often considered as an opportunistic infection in patients with impaired immune systems such as transplant recipients, patients with acquired immune deficiency syndrome (AIDS), passengers, children, elderly and people with ophthalmic lenses (Omalu et al., 2006). In recent decades, several new species of microsporidia were identified in AIDS patients that including *E. bieneusi* in 1985, *E. hellem* in 1991 and *Septata intestinalis* in 1993 (Weber et al., 1994). The microorganisms can be affect all body organs and cause spectrum symptoms such as myositis, keratoconjunctivitis, sinusitis, hepatitis and disseminated infections (Omalu et al., 2006) and other clinical signs consist of severe weight loss, chronic diarrhea, nausea and confusion (Lindsay and Weiss, 2004). *E. bieneusi* and *Encephalitozoon* spp. have been identified in animals that it is implying a zoonotic nature of these microorganisms, but valid proofs for transmission from these animals to human are lacking (Deplazes et al., 2000). Probably, many human microsporidiosis have zoonotic origin and transferred by water contaminated with animal stool. However, human to human transmission

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has also been described (Samie et al., 2007).

Studies in Iran, on microsporidiosis in susceptible groups have been performed recently, including prevalence studies in liver transplant recipient patients (Agholi et al., 2013), animals (Jamshidi et al., 2012), and HIV + /AIDS patients (Mirjalali et al., 2015) as well as with one study reporting on the prevalence of *E. bieneusi* in pigeons (Pirestani et al., 2013) but the current study is a novel research in southwest of Iran. Opportunistic infections are one of the major factors in mortality of patients with AIDS. Although, microsporidia are self-limited in immunocompetent hosts, but these parasites can be life-threatening in people with immune system dysfunction, especially in AIDS (Franzen and Müller, 1999; Samie et al., 2007). For this reason, due to the increasing prevalence of parasitic infections and immunodeficiency diseases; the aim of the study is to evaluate molecular identification of *Enterocytozoon bieneusi* and *Encephalitozoon* spp. in immunodeficient patients in Ahvaz, southwest of Iran.

2. Methods

2.1. Study area

Ahvaz is a city in the center of Khuzestan province in Southwest of Iran. The city is 375 square kilometers and its population has been reported as 1,425,891 until 2006. The city has a desert climate with temperatures above 50 °C that is one of the warmest cities in the world. The average annual rainfall is about 230 mm. According to report by the World Health Organization (WHO), Ahvaz has the most polluted climate in the world (Safi et al., 2016).

2.2. Sample collection

Initially, 310 stool samples were collected from immunodeficient patients referred to health centers and blood transfusion organization of Khuzestan province, Southwest of Iran. At first, the purpose and nature of the study were told to the individuals. The next step, stool samples were collected among them (Safi et al., 2016). Subjects were screened according to the inclusions and exclusions criteria. Inclusion criteria were: non-smoker, no alcohol usage. Exclusion criteria were: history of inflammatory diseases, diabetes, cardiovascular disease, thyroid, gastrointestinal, respiratory disorders, autoimmune diseases, and inflammatory status.

2.3. Ethical aspects

All experimental procedures were approved by the Ethics Committee of Jundishapur University of Medical Sciences (with OG-91109 code).

2.4. Preparing samples

The samples were collected and were transferred Department of Parasitology, Ahvaz Jundishapur University of Medical Sciences. Then, a part of the samples was used for smear preparation and staining. The rest of the sample was mixed with twice the volume of potassium dichromate 2.5% and were kept at 4 °C (Cama et al., 2007).

2.5. Samples examination by staining

All stool samples were stained by modified trichrome (weber). At first, the slides were fixed with methanol. Then, the samples were placed in trichrome color for 240 min. After decolorization with acid-alcohol, and washing with 95% ethanol, the slides were placed in absolute ethanol. At the next stage, to identify the spores, the slides were examined by an optical microscope at magnification of x100 together with immersion oil. The positive samples were identified by dorsal vacuoles in microsporidia spore (Pirestani et al., 2011a,b).

Table 1
The primary and secondary primers used for multiplex/nested PCR (Katzwinkel-Wladarsch et al., 1996).

The primary primers	The secondary primers
MSP-1: *TGAATGKGTCCCTGT*	MSP-3:*GGAATTCACACCGCCCGT C(A,G)(C,T) TAT*
MSP-2A: *TCACTCGCCGCTACT*	MSP-4A:*CCAAGCTTATGCTTAAGT (C,T) (A,C)AA(A,G)GGGT*
MSP-2B: *GTTCATTCGCACTACT*	MSP-4B: *CCAAGCTTATGCTTAAGTCCAGGGAG*

2.6. Extraction of DNA

The DNA was extracted by DNA extraction kits for stool (Bioneer) and the extracted DNA was stored at −20 °C. This kit was consist of spin column that the parasite DNA was absorbed by the column and after twice washing with special buffers, the purified DNA was obtained.

2.7. Molecular detection

The extracted DNA was examined by multiplex/nested PCR test that the microsporidial genera of *Enterocytozoon* and *Encephalitozoon* were identified by this method. At this stage, we used from specific primers that were designed by Katzwinkel et al. (Katzwinkel-Wladarsch et al., 1996). These primers were designed based on small subunit ribosomal RNA (16S rRNA) gene that the primers were used for the identification of different species of microsporidia. The primers were purchased from Bioneer Company and were stored at −20 °C. Table 1 indicates the primary and secondary primers used for multiplex/nested PCR. The amplified fragment length by the primers was 500 bp and 300 bp for the microsporidial genera of *Enterocytozoon* and *Encephalitozoon*, respectively. At first, the samples were examined with the primary and secondary primers by Multiplex/nested PCR method. Then, for differentiating the species of *Encephalitozoon*, the products of Multiplex/nested PCR were explored by RFLP method using the restriction enzyme of Mnl1.

2.8. Sequencing

For genotyping, the positive samples of RFLP were sequenced by Bioneer Company (Daejeon, South Korea). Afterwards, the specified sequence was compared against the sequence of the registered isolates available in the GenBank library (NCBI) and homology between them was examined by BLAST software. Finally according to 54 nucleotide sequences, the phylogenetic tree was drawn using the MEGA (version 7) software and Neighbor-Joining method (Fig. 4).

2.9. Statistical analysis

SPSS statistical software of version 16 was used for the data analysis and Chi-square test for significance differences. The P-value less than 0.05 were considered significant.

3. Results

3.1. Staining

Fig. 1 indicates microsporidia spore in the stool specimens of immunodeficient patients. Of 310, 93 samples were suspected positive for microsporidia spore by the staining with modified trichrome (weber) that 36, 34, 21, and 2 cases were observed in AIDS, hematologic malignancy, hemodialysis and kidney transplant patients, respectively. Of 93 positive samples of staining, 69 cases were observed positive by the molecular detection with multiplex/nested PCR method.

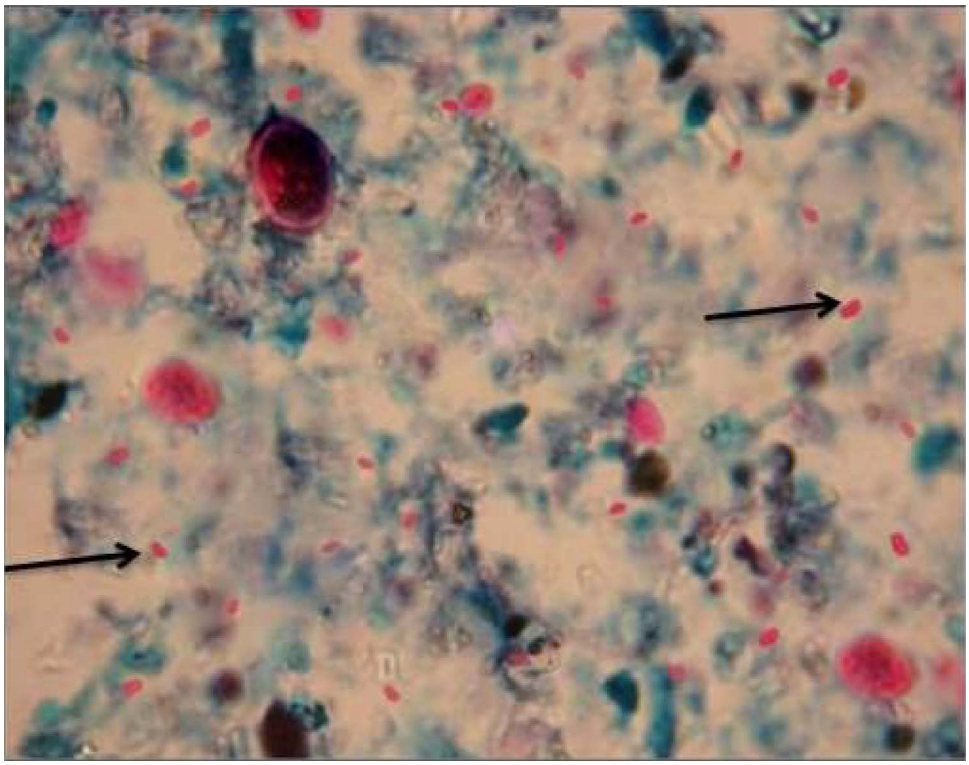

Fig. 1. *Microsporidia* spore in the stool sample that was stained by modified trichrome (weber), the slides were examined by optical microscope at magnification of x100.

Also, other parasites were observed in the samples that including *Blastocystis hominis*, *Giardia lamblia, Entamoeba coli* and *Cryptosporidium* species.

3.2. Molecular identification

Fig. 2 shows electrophoresis of the SSU rRNA gene PCR products on Agarose gel 2% and Fig. 3 indicates electrophoresis of the RFLP products on agarose gel 3%. Also, Table 2 shows the results of molecular analysis and genotyping of the fecal samples of immunodeficient patients in Ahvaz, southwest of Iran. Based on the results, of 310, 88 samples were positive by the multiplex/nested-PCR test that of 88, 62 samples were positive for *E. bieneusi*. Also, 26 were detected as *Encephalitozoon* species that including 3 *E. cuniculi*, 19 *E. intestinalis* and 4 *E. hellem*. Of 102 AIDS patients, 37 were positive that 23 patients had $CD_4^<$ 200 microliter and 13 patients had $CD_4^>$200 microliter. The results of other patients demonstrated in Table 2. No significant differences were found between the different groups of patients with immunodeficiency (AIDS, hemodialysis, hematologic malignancy and tissue transplantation) and microsporidiosis ($P > .05$).

3.3. Sequencing and genotyping

According to Table 2, Of 62 positive samples of *E. bieneusi,* 45, 16 and 1 specimens were detected as genotype D, M and WL11, respectively. Also, Of 3 positive specimens of *E. cuniculi,* 1 and 2 samples were identified as genotype I and II, respectively. All *E. hellem* samples were included genotype 1A. The data of nucleotide sequences reported in the

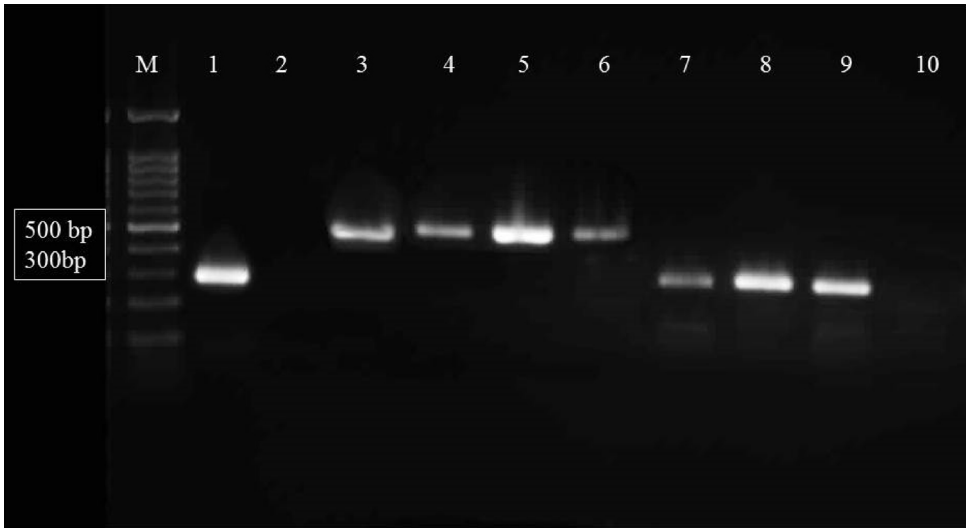

Fig. 2. Electrophoresis of the SSU rRNA gene PCR products on agarose gel 2%, M: 100 bp molecular marker, sample 1: *Encephalitozoon intestinalis* positive control, sample 2: Negative control, sample 3: *Enterocitozoon bineusi* positive control, samples 4–6: Positive samples for *E.bineusi* and samples7-9: Positive samples for *E.intestinalis.*

Fig. 3. Electrophoresis of the RFLP products on agarose gel 3%, M: 100 bp molecular marker, sample 1: *Encephalitozoon* spp. Positive control, sample 2: Negative control, sample 3: *Entrocitozoon bineusi* positive control, sample 4: *Encephalitozoon intestinalis* (160 and 60 bp), sample 5: *Encephalitozoon hellem* (180 and 80 bp), sample 6: *Encephalitozoon cuniculli* (210 and 90 bp).

paper are available in GenBank at accession numbers KY859460-KY859513. Also, Fig. 4 shows phylogenetic analysis of ITS sequences of microsporidia species isolates recovered from immunodeficient patients in Ahvaz, southwest of Iran.

4. Discussion

With improvement of molecular diagnostic methods, epidemiological knowledge of microsporidia infections has been developed in particular in the last decade (Pirestani et al., 2013). Reports related to microsporidia infections in patients with chronic diarrhea are increasing and its prevalence has reached 22% to 32% in developing countries (Ignatius et al., 1997; Samie et al., 2007). Accordingly in this study, we investigated microsporidiosis in immunodeficient patients in Ahvaz, southwest of Iran. Since, these microorganisms can be life-threatening in immunodeficient patients, especially in AIDS; these patients were selected for the study. In this research, we used from modified trichrome staining method based on the studies of Ryan et al. (Ryan et al., 1993). In the staining, the background color of blue aniline is stable and it has better contrast with other bacterial and fungal agents (Ryan et al., 1993). On the other hand, the PCR technique is a successful method that can be detect microsporidiosis with small numbers of spores in the clinical and environmental samples. According to the various studies, the detection threshold of microsporidia in fecal samples is 100 and 10,000–1000,000 spores per gram of feces by PCR and light microscopy, respectively. It represents the high sensitivity of PCR in comparison with the staining methods (Garcia, 2002; Müller et al., 2001).

Enterocytozoon bieneusi is the most common microsporidial species isolated from people with immunodeficiency and even immunocompetent individuals (Abdelmalek et al., 2011; Mirjalali et al., 2015). In the current study, also, this microorganism had the highest prevalence in immunodeficiency patients. Consistent with these results, in 2006, Leelayoova et al. in Thailand indicated that the highest prevalence of genotypes of *E. bieneusi* was related to genotype D (36.4%) in fecal samples of AIDS patients. The phylogenetic analysis of the data suggested the zoonotic importance of *E. bieneusi* (Leelayoova et al., 2006). Also, genotype D has been suggested as the most common genotype in most investigations (Thellier and Breton, 2008). So far, only genotype D has been detected in transplantation patients, in Iran (Agholi et al., 2013) but in our study, 1 case genotype M detected in the transplantation patients of bone marrow. Our results besides other investigations show that genotype D in almost immunodeficient patients could be associated with the widespread nature of the genotype. Also, the number of transplantation individuals registered in our research was lower than other groups such as AIDS, hemodialysis and leukemia. Also, our findings show that of 62 positive samples of *E. bieneusi,* 16 cases were detected as genotype M. Previous studies showed that genotype M was only in animals (Pirestani et al., 2011a,b) but in the study, we observed this genotype in immunodeficient patients that the results suggest the zoonotic potential of this genotype.

On the other hand, our findings show that 26 cases were detected as *Encephalitozoon* species that including 3 *E. cuniculi*, 19 *E. intestinalis* and 4 *E. hellem*. *Encephalitozoon intestinalis* is the second most common microsporidial species infecting human (Deplazes et al., 2000) that this is consistent with our results as well as in several studies, the parasite besides *E. hellem* were detected in immunodeficient patients (Deplazes et al., 2000).The studies on *E. cuniculi* indicated that these microorganisms were detected in 7 AIDS patients from Switzerland (Deplazes et al., 1996; Weber et al., 1997), two cases from the USA (De Groote et al., 1995; Mertens et al., 1997), and in one each from Germany (Franzen et al., 1995), the United Kingdom (Hollister et al., 1995) and Italy (Rossi et al., 1998), that prove the infection of *E. cuniculi* for immunodeficient patients. Also, immunodeficient patients were detected to be infected with the 'dog strain' in the Americas and with the 'rabbit strain' in Europe. In 1 AIDS patient from the UK, the *E. cuniculi* 'dog strain' was found (Hollister et al., 1996) that the results suggest the zoonotic nature of these microorganisms.

Eventually, this study is important in terms of health; because the opportunistic pathogens such as human microsporidiosis were isolated

Table 2
The results of molecular analysis and genotyping of the fecal samples of immunodeficient patients in Ahvaz, southwest of Iran.

Patients	Number	*E. bieneusi (Genotype)*	*Encephalitozoon*	*E. intestinalis*	*E. cuniculi* (Genotype)	*E. hellem* (Genotype)
AIDS	102	21 (D) 8 (M)	8	6	1 (II)	1 (1A)
Leukemia	68	12 (D)	2	2	0	0
Lymphoma	12	1 (M)	0	0	0	0
Hodgkin lymphoma	20	5 (D) 3 (M)	1	0	0	1 (1A)
Hemodialysis	87	6 (D) 3 (M) 1 (WL11)	13	9	1 (I) 1 (II)	2 (1A)
Bone marrow transplant	12	1 (M)	2	2	0	0
Kidney transplant	8	1 (D)	0	0	0	0
Liver transplant	1	0	0	0	0	0
Total	310	62	26	19	3	4

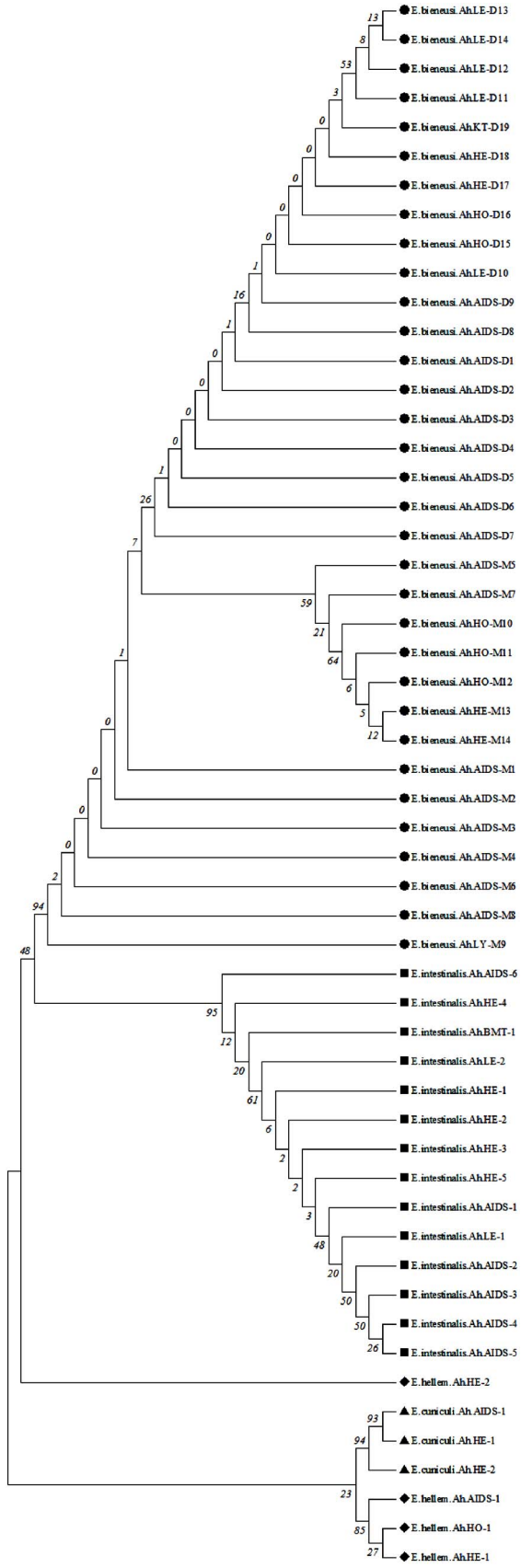

Fig. 4. Phylogenetic analysis of ITS sequences of microsporidia species isolates recovered from immunodeficient patients in Ahvaz, southwest of Iran. According to 54 nucleotide sequences, the phylogenetic tree was drawn using the MEGA (version 7) software and Neighbor-Joining method. AIDS: acquired immune deficiency syndrome, BMT: Bone marrow transplant, HE: Hemodialysis, HO: Hodgkin lymphoma, LE: Leukemia, LY: Lymphoma, KT: Kidney transplant.

from patients with impaired immune systems in Ahvaz, southwest of Iran. It has been reported that *E. bieneusi* and *Encephalitozoon* spp. have been identified in animals (Deplazes et al., 2000) and birds (Yee et al., 1991). Due to the zoonotic potential of the microsporidia as well as because of the close relationship of these animals with humans, the animals can be an important source of pollution. Therefore, in order to the design of appropriate prevention programs for the parasite, it is essential that the high-risk individuals such as patients with impaired immune systems should be receiving the accurate information about the risk of direct relationship with these animals. Also, it is recommended to researchers to examine the different hosts such as the domestic and wild animals and the role of these animals in infecting high-risk individuals and other people. Hence, it is need to examine the whole range of epidemiological and molecular studies on the hosts. This study is one of the rare studies that have investigated microsporidiosis in the different groups of patients with immunodeficiency (AIDS, hemodialysis, hematologic malignancy and tissue transplantation) and the current study is a novel research in southwest of Iran. Due to the increasing prevalence of parasitic infections and immune deficiency diseases such as patients with acquired immune deficiency syndrome (AIDS), transplant recipients; the detection and species determination of the parasites seems essential for the adoption of health strategies.

5. Conclusion

Our findings revealed a relatively high prevalence of microsporidia species in immunodeficient patients. The highest risk of this infection is at individuals with impaired immune systems that it can be life-threatening in people with immune system dysfunction. It is essential that the high-risk people should be receiving the information about the risk of direct contact with infected individuals and animals.

Conflict of interest

The authors declare no conflict of interests.

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References

Abdelmalek, R., Anane, S., Chabchoub, N., Essid, R., Aoun, K., Chaabéne, T.B., Bouratbine, A., 2011. Microsporidia and cryptosporidia coinfection in an HIV-infected newborn. Arch. Pediatr. 18 (5), 562–564.

Agholi, M., Hatam, G.R., Motazedian, M.H., 2013. Microsporidia and coccidia as causes of persistence diarrhea among liver transplant children: incidence rate and species/genotypes. Pediatr. Infect. Dis. J. 32 (2), 185–187.

Cama, V.A., Pearson, J., Cabrera, L., Pacheco, L., Gilman, R., Meyer, S., Xiao, L., 2007. Transmission of Enterocytozoon bieneusi between a child and guinea pigs. J. Clin. Microbiol. 45 (8), 2708–2710.

De Groote, M.A., Visvesvara, G., Wilson, M.L., Pieniazek, N.J., Slemenda, S.B., Leitch, G.J., Reves, 1995. Polymerase chain reaction and culture confirmation of disseminated Encephalitozoon cuniculi in a patient with AIDS: successful therapy with albendazole. J. Infect. Dis. 171 (5), 1375–1378.

Deplazes, P., Mathis, A., Baumgartner, R., Tanner, I., Weber, R., 1996. Immunologic and molecular characteristics of Encephalitozoon-like microsporidia isolated from humans and rabbits indicate that Encephalitozoon cuniculi is a zoonotic parasite. Clin. Inf. Dis. 22 (3), 557–559.

Deplazes, P., Mathis, A., Weber, R., 2000. Epidemiology and Zoonotic Aspects of Microsporidia of Mammals and Birds *Cryptosporidiosis* and *Microsporidiosis*. Karger Publisherspp. 236–260.

Franzen, C., Müller, A., 1999. Molecular techniques for detection species differentiation, and phylogenetic analysis of microsporidia. Clin. Microbiol. Rev. 12 (2), 243–285.

Franzen, C., Schwartz, D.A., Visvesvara, G.S., Müller, A., Schwenk, A., Salzberger, B., Diehl, 1995. Immunologically confirmed disseminated, asymptomatic Encephalitozoon cuniculi infection of the gastrointestinal tract in a patient with AIDS.

Clin. Infect. Dis. 21 (6), 1480–1484.
Garcia, L.S., 2002. Laboratory identification of the microsporidia. J. Clin. Microbiol. 40 (6), 1892–1901.
Hollister, W.S., Canning, E.U., Colbourn, N.I., Aarons, E.J., 1995. Encephalitozoon cuniculi isolated from the urine of an AIDS patient, which differs from canine and murine isolates. J. Eukaryot. Microbiol. 42 (4), 367–372.
Hollister, W.S., Canning, E.U., Anderson, C.L., 1996. Identification of microsporidia causing human disease. J. Eukaryot. Microbiol. 43 (5).
Ignatius, R., Lehmann, M., Miksits, K., Regnath, T., Arvand, M., Engelmann, E., Wagner, J., 1997. A new acid-fast trichrome stain for simultaneous detection of Cryptosporidium parvum and microsporidial species in stool specimens. J. Clin. Microbiol. 35 (2), 446–449.
Jamshidi, S., Tabrizi, A.S., Bahrami, M., Momtaz, H., 2012. Microsporidia in household dogs and cats in Iran; a zoonotic concern. Vet. Parasitol. 185 (2), 121–123.
Katzwinkel-Wladarsch, S., Lieb, M., Heise, W., Löscher, T., Rinder, H., 1996. Direct amplification and species determination of microsporidian DNA from stool specimens. Trop. Med. Int. Health 1 (3), 373–378.
Leelayoova, S., Subrungruang, I., Suputtamongkol, Y., Worapong, J., Petmitr, P.C., Mungthin, M., 2006. Identification of genotypes of Enterocytozoon bieneusi from stool samples from human immunodeficiency virus-infected patients in Thailand. J. Clin. Microbiol. 44 (8), 3001–3004.
Lindsay, D.S., Weiss, L.M., 2004. Opportunistic Infections: Toxoplasma, Sarcocystis, and Microsporidia, vol. 9 Springer Science & Business Media.
Müller, A., Bialek, R., Kämper, A., Fätkenheuer, G., Salzberger, B., Franzen, C., 2001. Detection of microsporidia in travelers with diarrhea. J. Clin. Microbiol. 39 (4), 1630–1632.
Mertens, R.B., Didier, E.S., Fishbein, M.C., Bertucci, D.C., Rogers, L.B., Orenstein, J.M., 1997. Encephalitozoon cuniculi microsporidiosis: infection of the brain, heart, kidneys, trachea, adrenal glands, and urinary bladder in a patient with AIDS. Mod. Pathol. 10 (1), 68–77.
Mirjalali, H., Mirhendi, H., Meamar, A.R., Mohebali, M., Askari, Z., Mirsamadi Rezaeian, E.S.M., 2015. Genotyping and molecular analysis of Enterocytozoon bieneusi isolated from immunocompromised patients in Iran. Infect. Genet. Evol. 36, 244–249.
Omalu, I., Duhlinska, D., Anyanwu, G., Parn, V., Inyama, P., 2006. Human microsporidial infections. Online J. Health Allied Sci. 3 (2).
Pirestani, M., Sadraei, J., Forouzandeh, M., 2011a. Molecular characterization of zoonotic isolates of Enterocytozoon bieneusi in Iran. Feyz J. Kashan Univ. Med. Sci. 16 (1).
Pirestani, M., Sadraei, J., Forouzandeh Moghadam, M., 2011b. Detection and genotyping of human-associated microsporidia in pigeon Columba livia of Tehran in 2010. Modares J. Med. Sci.: Pathobiol. 14 (3), 15–24.
Pirestani, M., Sadraei, J., Forouzandeh, M., 2013. Molecular characterization and genotyping of human related microsporidia in free-ranging and captive pigeons of Tehran Iran. Infect. Genet. Evol. 20, 495–499.
Rossi, P., La Rosa, G., Ludovisi, A., Tamburrini, A., Morales, M.G., Pozio, E., 1998. Identification of a human isolate of Encephalitozoon cuniculi type I from Italy? Int. J. Parasitol. 28 (9), 1361–1366.
Ryan, N.J., Sutherland, G., Coughlan, K., Globan, M., Doultree, J., Marshall, J., Dwyer, B., 1993. A new trichrome-blue stain for detection of microsporidial species in urine, stool, and nasopharyngeal specimens. J. Clin. Microbiol. 31 (12), 3264–3269.
Safi, M., Tavalla, M., Mardani, M., Afrisham, R., 2016. Prevalence of intestinal parasitic infections among applicants for health cards attending Ahvaz East Health Center during 2012–2013. Asian Pac. J. Trop. Dis. 6 (2), 151–154.
Sak, B., Kašičková, D., Kváč, M., Květoňová, D., Ditrich, O., 2010. Microsporidia in exotic birds: intermittent spore excretion of Encephalitozoon spp. in naturally infected budgerigars (Melopsittacus undulatus). Vet. Parasitol. 168 (3), 196–200.
Samie, A., Obi, C., Tzipori, S., Weiss, L., Guerrant, R., 2007. Microsporidiosis in South Africa: PCR detection in stool samples of HIV-positive and HIV-negative individuals and school children in Vhembe district, Limpopo Province. Trans. R. Soc. Trop. Med. Hyg. 101 (6), 547–554.
Thellier, M., Breton, J., 2008. Enterocytozoon bieneusi in human and animals: focus on laboratory identification and molecular epidemiology. Parasite 15 (3), 349–358.
Weber, R., Bryan, R.T., Schwartz, D.A., Owen, R.L., 1994. Human microsporidial infections. Clin. Microbiol. Rev. 7 (4), 426–461.
Weber, R., Deplazes, P., Flepp, M., Mathis, A., Baumann, R., Sauer, B., Lüthy, R., 1997. Cerebral microsporidiosis due to Encephalitozoon cuniculi in a patient with human immunodeficiency virus infection. New Engl. J. Med. 336 (7), 474–478.
Yee, R.W., Tio, F.O., Martinez, J.A., Held, K.S., Shadduck, J.A., Didier, E.S., 1991. Resolution of microsporidial epithelial keratopathy in a patient with AIDS. Ophthalmology 98 (2), 196–201.